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DOCUMENT-IDENTIFIER: US 5709859 A
TITLE: Mixed specificity fusion proteins

US PATENT NO. (1):
5709859

Brief Summary Text (15):
Although a vast majority of reports dealt with the inhibition of various adhesion-dependent functions of leukocytes in vitro by mAb directed at beta2 integrins, a few elegant studies have been carried out with anti-CD18 mAb in vivo in experimental animals and also in man. In mice, antibodies to the beta2 integrins have been shown to inhibit migration of lymphoid cells into lymph nodes and Peyer's patches, recruitment of monocytes and neutrophils to thioglycollate-elicited peritoneal exudates and thus, reducing the severity of peritonitis (Patarroyo, M., and Makgoba, M. W., 1989, Scand. J. Immunol. 30:129-164). Using isolated perfused lungs as a model system, pulmonary injury caused by phorbol ester-activated human neutrophils could be attenuated by mAb to beta2 integrin (Patarroyo, M., and Makgoba, M. W., 1989, Scand. J. Immunol. 30:129-164). Similarly, accumulation of neutrophils and proteinaceous contents of the plasma in inflamed skin lesions were shown to be reduced by anti-CD18 mAb, perhaps due to inhibition of both the adherence to endothelium and subsequent migration across into the extravascular tissue (Smith, C. W., Rothlein, R., Hughes, B. J., Mariscalco, M. M., Rudloff, H. E., Schmalsteig, F. C., and Anderson, D. C., 1988, J. Clin. Invest. 82:1746-1756; Smith, C. W., Marlin, S. D., Rothlein, R., Toman, C., and Anderson, D. C., 1989, J. Clin. Invest. 83:2008-2017; Vedder, N. B., Winn, R. K., Rice, C. L., Chi, E. Y., Arfors, K. E., and Harlan, J. M., 1990, Proc. Natl. Acad. Sci. USA, 87:2643-2646).

Brief Summary Text (17):
Mab 60.3 recognizes an epitope on the CD18 (beta2 integrin) molecule (Beatty, P. G., Ledbetter, J. A., Martin, P. J., Price, T. H., and Hansen, J. A., 1983, J. Immunol. 131:2913-2918) which is a constituent of all the three beta2 integrins (CD11a, CD11b, CD11c) critically involved in all functions mediated via beta2 integrins (Springer T. A., 1990, Nature 425-434; Patarroyo, M., and Makgoba, M. W., 1989, Scand. J. Immunol. 30:129-164; Moller, G. Editor, 1990,Immunol. Rev. 114:1-217; Smith, C. W., Rothlein, R., Hughes, B. J., Mariscalco, M. M., Rudloff, H. E., Schmalsteig, F. C., and Anderson, D. C., 1988, J. Clin. Invest. 82:1746-1756; Vealder, N. B., Winn, R. K., Rice, C. L., Chi, E. Y., Arfors, K.-E., and Harlan, J. M., 1990, Proc. Natl. Acad. Sci. USA, 87:2643-2646). Hence, mAb 60.3 can be used to inhibit adhesion-dependent functions of leukocytes which differentially make use of distinct beta2 integrins. This mAb is one of the first, if not the first, anti-CD18 mAb described (Patarroyo, M., and Makgoba, M. W., 1989, Scand. J. Immunol. 30:129-164; Beatty, P. G., Ledbetter, J. A., Martin, P. J., Price, T. H., and Hansen, J. A., 1983, J. Immunol. 131:2913-2918) and its use in both in vitro and in vivo studies has been well-documented (Patarroyo, M., and Makgoba, M. W., 1989, Scand. J. Immunol. 30:129-164; Moller, G. Editor, 1990, Immunol. Rev. 114:1-217). This mAb is highly regarded among anti-CD18 antibodies in its ability to inhibit a plethora of leukocyte functions. For application in humans, however, the use of a mouse antibody presents several immunologic difficulties. The present inventors have therefore produced novel mixed specificity fusion proteins derived from human protein genes which are applicable for use in inhibiting inflammation and metastasis in humans.

Brief Summary Text (20):
One class of molecules of the present invention are immunoglobulin-like fusion proteins having a mixed specificity containing such binding regions. The immunoglobulin constant

region of these fusion proteins can substantially correspond to a constant region of IgG. Binding regions of the fusion proteins can comprise binding portions of the extracellular domains of cell surface receptors, such as ELAM-1, GMP140, and ICAM-1. Specific fusion proteins contemplated by the present invention include a fusion protein of a human IgG constant region attached to regions of the extracellular domains of ELAM-1 and GMP140, a fusion protein of an IgG constant region attached to regions of the extracellular domains of ICAM-1 and ELAM-1, a fusion protein of an IgG constant region attached to regions of the extracellular domains of ELAM-1 and VCAM-1, and a fusion protein of an IgG constant region attached to regions of the extracellular domains of ICAM-1 and GMP140.

Detailed Description Text (9):
In a preferred embodiment, the fusion proteins of the present invention are produced by fusing the cDNA fragments encoding the extracellular domains of the endothelial and granulocyte surface receptors responsible for neutrophil-endothelium binding, such as ICAM-1/ICAM-2, VCAM-1, ELAM-1 and GMP140, to a genomic fragment encoding the human IgG constant region. Combinations of these constructs are then transfected into mammalian cells. The mixed specificity receptor-immunoglobulin fusion proteins are thereby assembled in these cells and secreted side by side with the single specificity immunoglobulin fusion proteins. In the present invention mixed specificity fusion proteins, such as ICAM-1/ELAM-1, ICAM-1/ GMP-140, VCAM-1/GMP-140 etc., have been produced and can be tested alone and in combination for their ability to bind neutrophils and alleviate reperfusion injury.

Detailed Description Text (10):
Fusion proteins of the present invention are preferably produced by the fusion of human proteins and, as such, would be less immunogenic than non-human monoclonal antibodies that may have related specificity to one or more adhesion molecules. The multiple specificity of these fusion proteins enables the simultaneous binding of several of the neutrophil proteins responsible for neutrophil-endothelial binding, and thus will be potent blockers of the neutrophil-endothelial adhesion that is associated with reperfusion injury and inflammation.

Detailed Description Text (18):
Expression plasmids containing cDNA fragments encoding the complete extracellular domain of ELAM-1 and the four amino terminal domains of the GMP140 protein fused to a genomic fragment encoding the human IgG constant region were mixed in equal amounts and cotransfected into COS monkey cells by the DEAE-dextran method of Seed, B. and Aruffo, A., 1987, Proc. Natl. Acad. Sci. USA, 84:3365-3369.

CLAIMS:

6. The heterodimeric molecule according to claim 5 wherein said immunoglobulin constant region substantially corresponds to a constant region of human IgG.

18. The composition according to claim 17 wherein said immunoglobulin constant region substantially corresponds to a constant region or human IgG.

Brief Summary Text (56):
The present invention facilitates the large scale production of immunoglobulin-like domains, including those derived from human sources, which
may be employed in a wide variety of embodiments. These include their use in in vitro mutagenesis studies and in high resolution structural
analyses, such as NMR and X-ray crystallography. Fc-hinge and Fc domain analyses have allowed the region involved in antibody catabolism to be
delineated, showing that residues isoleucine (ile) 253, histidine (his) 310, his 435 and his 436 are important. Recombinant fragments, domains,
or even subfragments thereof, may be used for mapping the Fc residues which are functionally important in binding to FcRn. Residues of
recombinant Fc fragments may be altered, prior to expression as soluble proteins as disclosed herein, or on the surface of bacteriophage
(McCafferty et al., 1990), and mutants binding with higher affinity to FcRn may be screened, or selected for, using solid surfaces coated with
FcRn or FcRn in solution. The preferred method is to use FcRn in solution and then to capture FcRn:bacteriophage complexes on beads.

Brief Summary Text (61):
In light of the previous discussion, the present invention may be described in certain broad aspects as a composition comprising a mutant IgG
molecule having an increased serum half-life relative to IgG, and wherein said mutant IgG molecule has at least one amino acid substitution in
the Fc-hinge region. The IgG may be any IgG molecule and is in certain embodiments, preferably a human IgG.

Detailed Description Text (23):
The production of the IgG1 Fc-hinge or Fc fragment in E. coli has allowed the important residues of this region involved in controlling antibody
stability and catabolism in vivo to be elucidated. These results are described in Example 8. Furthermore, following the present invention, human
Fc domains may now be produced in E. coli, allowing further detailed studies of the human protein. Additionally, the bacterial secretion of Fc or
Fc-hinge domains, or Fc or Fc-hinge domain:fusion proteins, whether of murine or human origin, is envisioned to provide a convenient,
economically attractive and rapid route for the production of novel proteins that have long serum persistence.